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SULFUR BUDGET OF LAKE SHELBYVILLE, ILLINOIS, AND THE EFFECTS OF SULFIDES UPON CHAOBORUS

By WARREN U. BRIGHAM
and SARAH R. GNILKA

ILLINOIS NATURAL HISTORY SURVEY
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

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Warren U. Brigham
Assistant Aquatic Biologist

and Sarah R. Gnilka
Research Assistant

Illinois Natural History Survey
Urbana, Illinois

FINAL REPORT

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Warren U. Brigham, Principal Investigator

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UNIVERSITY OF ILLINOIS
WATER RESOURCES CENTER
2535 Hydrosystems Laboratory
Urbana, Illinois 61801

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ABSTRACT

SULFUR BUDGET OF LAKE SHELBYVILLE, ILLINOIS, AND THE EFFECTS OF SULFIDES UPON CHAOBORUS

The sources and annual cycle of sulfur were studied in the Lake Shelbyville Basin, Illinois, from 6 April 1972 through 28 March 1973. Three sources of sulfur were identified: rainfall (3.34×10^4 kg year^{-1}, surface runoff (2.05×10^7 kg year^{-1}, and pollution (4.56×10^6 kg year^{-1}). Decomposition of innundated organic matter and solution of inorganic sulfur compounds from the innundated basin were considered one-time sources. Exchange between groundwater and the lake was considered to be in long-term equilibrium.

Significant relationships (0.001 level) between sulfate loading and discharge were detected. Regression analysis yielded an accurate means of calculating sulfate concentration from discharge measurements.

During the study year, 2.51×10^7 kg of sulfur entered Lake Shelbyville. Of this, 2.01×10^7 kg flowed out through the dam, 3.35×10^6 kg remained in the lake due to a net gain in water storage, and 1.65×10^6 kg was either assimilated by the biota or, in part, lost to the groundwater.

Green sulfur bacteria were isolated from lake samples during the study year. During the previous summer stagnation period, purple sulfur bacteria were detected accompanying noticeable concentrations of hydrogen sulfide.

Static bioassay studies of Chaoborus larvae yielded a minimum effect level for sulfide of 0.64 mg liter^{-1}(as S) for a 4-hour test. Pupae responded more rapidly to sulfide than did larvae. Relatively brief exposure to a low sulfide concentration (0.8 mg liter^{-1} as S) was often sufficient to kill or immobilize a substantial percentage of the test organisms.

Brigham, Warren U., and Sarah R. Gnilka
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Dr. Arnold Gnilka and Messrs. Matthew K. Butcher and Donald W. Dufford assumed much of the responsibility for sample collection and preliminary water analysis. Dr. Gnilka also participated in the bioassay study. Mr. Mark J. Wetzel isolated and cultured sulfur bacteria from Lake Shelbyville, and made his findings available to us.

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INTRODUCTION

Preliminary studies on the Kaskaskia River System, Illinois, indicated that sulfate concentrations in the Lake Shelbyville Basin (Table 1) were considerably higher than in similar portions of three closely associated river systems: the Sangamon to the north; the Embarras to the east; and the Little Wabash to the south. Reduction of sulfate and the decomposition of sulfur-containing organic matter in the hypolimnion of Lake Shelbyville during 1971 produced undesirable concentrations of hydrogen sulfide. Discharge of sulfide-bearing water through the dam at Shelbyville, Illinois, resulted in considerable degradation of air quality in the vicinity of the dam. In addition, several fish kills in Lake Shelbyville were suspected to be sulfide-caused.

These preliminary studies and observations indicated that the water resource potential of Lake Shelbyville was affected substantially by the dynamics of sulfur in the Kaskaskia River Basin. The purpose of this study was to identify the sources and investigate the annual cycle of sulfur in the Lake Shelbyville Basin. Particular emphasis was given to the sulfur balance of the lake, the role of bacteria in the sulfur cycle, and the toxicity of hydrogen sulfide to aquatic organisms.

Table 1. The number of determinations (followed by dash), mean, standard deviation (in parentheses), and range of measurements for various parameters measured from 10 stations in the Lake Shelbyville Basin, Illinois, from 6 April 1972 through 28 March 1973 (except for stations marked with asterisk, which are for the period 10 October 1971 through 30 September 1972, as reported by Brigham (1973)).

Station	Redox Potential (volts)	Hydrogen Ion (pH)	Water Temperature (C)
I	13-0.20(0.08) 0.11-0.34	19-7.9(0.2) 7.5-8.3	19-15.4(6.7) 2.0-24.0
II_0	14-0.21(0.07) 0.12-0.35	19-7.6(0.3) 7.6-9.0	19-17.9(8.8) 1.0-27.6
II_5	14-0.21(0.07) 0.12-0.37	19-8.1(0.3) 7.8-9.0	19-16.8(8.1) 1.0-26.0
II_{10}	14-0.21(0.06) 0.13-0.34	19-7.9(0.3) 7.4-8.4	19-14.7(6.9) 1.0-22.5
II_{15}	13-0.20(0.06) 0.10-0.33	18-7.8(0.3) 7.4-8.5	18-12.4(4.5) 1.0-19.0
II_{20}^*	-	1-8.3	1-3.2
III_0	12-0.19(0.09) 0.09-0.39	17-8.3(0.4) 7.6-8.8	17-16.5(9.0) 1.0-27.6
III_5	12-0.19(0.09) 0.08-0.39	17-8.1(0.3) 7.6-8.4	17-15.9(8.6) 1.0-25.9
III_{10}	12-0.20(0.08) 0.06-0.33	17-7.8(0.3) 7.4-8.2	17-14.2(7.3) 1.0-22.6
III_{15}^*	-	1-8.1	1-2.0
IV_0	11-0.17(0.07) 0.07-0.26	17-8.2(0.4) 7.4-8.5	17-17.5(9.6) 1.0-29.3
IV_5	10-0.16(0.06) 0.07-0.26	15-7.9(0.3) 7.5-8.5	15-16.3(8.1) 1.0-24.5

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Station	Redox Potential (volts)	Hydrogen Ion (pH)	Water Temperature (C)
V*	1-0.03	19-8.2(0.6) 7.3-9.5	19-20.0(9.8) 2.2-37.0
VI*	1-0.11	19-7.9(0.4) 7.2-8.4	19-16.9(9.4) 0.9-33.0
VII*	1-0.08	4-8.3(0.3) 8.1-8.7	4-14.0(11.7) 2.0-30.0
VIII*	1-0.06	19-8.0(0.5) 7.0-8.8	19-18.2(9.3) 0.9-29.2
IX*	1-0.10	19-7.9(0.3) 7.1-8.4	19-16.0(8.7) 0.6-29.5
X*	9-0.15(0.09) 0.01-0.25	152-7.9(0.3) 7.1-8.4	152-13.2(8.8) 0.0-30.8

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Station	Dissolved Oxygen (mg liter^{-1})	Iron, Total (mg liter^{-1})	Iron, Ferrous (mg liter^{-1})
I	19-10.5(2.5) 7.2-15.5	17-0.85(0.67) 0.08-2.30	16-0.09(0.10) 0.00-0.35
II_0	19-9.4(3.1) 3.2-15.0	17-0.39(0.29) 0.08-1.00	16-0.08(0.07) 0.00-0.20
II_5	19-7.2(3.1) 2.2-13.6	16-0.39(0.28) 0.10-1.20	16-0.08(0.06) 0.00-0.20
II_{10}	19-4.5(4.4) 0.1-12.8	17-0.44(0.28) 0.06-1.25	16-0.06(0.06) 0.00-0.25
II_{15}	18-3.4(4.5) 0.1-12.8	17-0.59(0.42) 0.08-0.88	15-0.06(0.04) 0.00-0.13
II_{20}*	1-10.2	1-0.50	-
III_0	17-9.8(2.4) 3.5-13.3	15-0.42(0.26) 0.00-1.08	14-0.06(0.08) 0.00-0.30
III_5	17-8.7(2.6) 3.5-12.9	15-0.46(0.30) 0.00-1.25	14-0.07(0.08) 0.00-0.30
III_{10}	17-5.0(4.5) 0.2-12.9	14-1.43(1.49) 0.38-4.70	14-0.14(0.11) 0.00-0.25
III_{15}*	1-13.5	1-0.80	-
IV_0	17-10.5(3.1) 4.2-17.0	15-0.90(0.61) 0.20-2.35	13-0.08(0.11) 0.00-0.35
IV_5	15-4.7(4.3) 0.2-13.2	13-1.34(0.91) 0.08-3.25	12-0.09(0.07) 0.02-0.20
V*	19-11.6(2.3) 7.9-16.4	10-0.80(0.39) 0.33-1.60	-
VI*	19-8.8(3.3) 3.9-16.0	10-1.45(0.85) 0.08-2.70	-
VII*	4-10.7(3.6) 5.3-13.0	4-1.43(1.12) 0.51-3.00	-

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Station	Dissolved Oxygen (mg liter^{-1})	Iron, Total (mg liter^{-1})	Iron, Ferrous (mg liter^{-1})
VIII*	19-9.3(2.6) 6.2-15.4	10-2.02(1.8) 0.19-5.70	-
IX*	19-8.8(3.4) 3.9-15.4	10-1.56(1.09) 0.16-3.85	-
X*	34-8.9(2.4) 4.4-16.6	35-2.73(1.41) 0.25-7.10	6-0.23(0.21)

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Station	Sulfate (mg liter^{-1}as S)	Sulfite (mg liter^{-1}as S)	Sulfide[1] (mg liter^{-1}as S)
I	19-20.7(16.6) 11.5-85	12-0.54(0.36) 0.36-1.2	
II_0	19-21.2(17.2) 13.0-88	12-0.45(0.27) 0.4-0.88	
II_5	19-22.1(17.6) 12.67-91	11-0.50(0.34) 0.36-1.0	
II_{10}	19-21.7(17.3) 12.67-88	12-0.47(0.30) 0.36-0.92	
II_{15}	18-20.5(18.4) 11.2-91	12-0.51(0.30) 0.36-0.80	
II_{20}*	1-35.0	-	
III_0	17-20.6(14.0) 12.17-71	10-0.46(0.33) 0.36-1.04	
III_5	17-24.6(22.0) 12.5-85	10-0.48(0.37) 0.32-1.20	
III_{10}	17-23.8(23.6) 12.00-92	10-0.50(0.35) 0.36-1.00	
III_{15}*	1-44.6	-	
IV_0	17-25.5(22.3) 11.0-89.0	9-0.62(0.71) 0.36-2.36	
IV_5	15-27.0(27.4) 11.33-99.0	9-0.50(0.43) 0.40-1.40	
V*	19-62.9(27.3) 27.5-144	-	
VI*	19-32.9(9.8) 17.8-54.8	-	
VII*	4-163(132) 71.5-354	-	

(continued on the next page)

Station	Sulfate (mg liter^{-1} as S)	Sulfite (mg liter^{-1} as S)	Sulfide[1] (mg liter^{-1} as S)
VIII*	19-37.8(9.6) 26.0-59.7	-	
IX*	19-40.0(7.5) 28-56.0	-	
X*	149-134.7(129.4) 27-710	-	

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Station	Bacterioviridine-654 (mg m^3)	Bacterioviridine-662 (mg m^3)	Bacteriochlorophyll-772 (mg m^3)
I	13-6.000(2.512) 1.340-10.880	13-10.170(3.917) 5.184-19.008	13-0.766(1.747) 0.000-6.333
II_0	13-8.424(5.417) 0.504-18.728	13-11.623(6.501) 1.008-23.184	13-0.886(2.804) 0.000-10.200
II_5	13-6.061(3.480 0.504-12.580	13-9.823(4.211) 1.296-17.352	13-1.152(2.767) 0.000-9.598
II_{10}	13-5.257(4.146) 0.000-17.068	13-7.950(6.132) 2.376-26.640	13-0.368(0.677) 0.000-2.344
II_{15}	13-6.944(7.215) 1.612-29.784	13-10.392(10.654) 0.944-44.496	13-0.878(1.676) 0.000-5.916
II_{20}*	-	-	-
III_0	11-8.949(4.842) 1.512-16.116	11-16.093(8.126) 3.850-32.180	11-2.357(6.673) 0.000-22.440
III_5	11-7.449(5.202) 0.000-13.668	11-12.273(6.285) 2.664-23.760	11-0.959(2.666) 0.000-8.976
III_{10}	10-7.316(5.330) 0.952-16.728	11-12.879(6.848) 0.648-23.760	11-1.444(3.374) 0.000-11.420
III_{15}*	-	-	-
IV_0	11-32.801(30.065) 0.000-104.856	11-54.162(54.675) 1.760-200.900	11-14.368(46.512) 0.000-154.600
IV_5	11-17.913(15.329) 0.000-37.497	10-33.759(23.462) 1.620-65.656	11-2.127(5.824) 0.000-19.580
V*	-	-	-
VI*	-	-	-
VII*	-	-	-

(continued on the next page)

Station	Bacterioviridine-654 (mg m^3)	Bacterioviridine-662 (mg m^3)	Bacteriochlorophyll-772 (mg m^3)
VIII*	-	-	-
IX*	-	-	-
X*	-	-	-

[1]Not detected during study.

METHODS

This study was conducted in the upper portion of the Kaskaskia River Basin in east-central Illinois (Fig. 1) from 6 April 1972 through 28 March 1973. Ten stations were located in and around Lake Shelbyville, Moultrie and Shelby Counties. These stations were visited biweekly during the period May through October and monthly during the remainder of the year with the exception of station X, which was sampled three times per week. Stations II through IV were deepwater sites and collections there consisted of a surface and a series of 5-meter-depth-interval grab samples plus oxygen-temperature profiles with measurements at 1 m intervals. All other stations were represented by surface grab samples.

Many parameters were measured on each of these visits as part of a water quality monitoring program on Lake Shelbyville. In addition to these parameters, several were added to elucidate sulfur dynamics in the watershed. Parameters to be discussed here include the following:

Bacteriochlorophyll 772 (mg m^{-3})

Bacterioviridines 654 and 662 (mg m^{-3})

Dissolved Oxygen (mg $liter^{-1}$)

Hydrogen Ion Concentration (as pH)

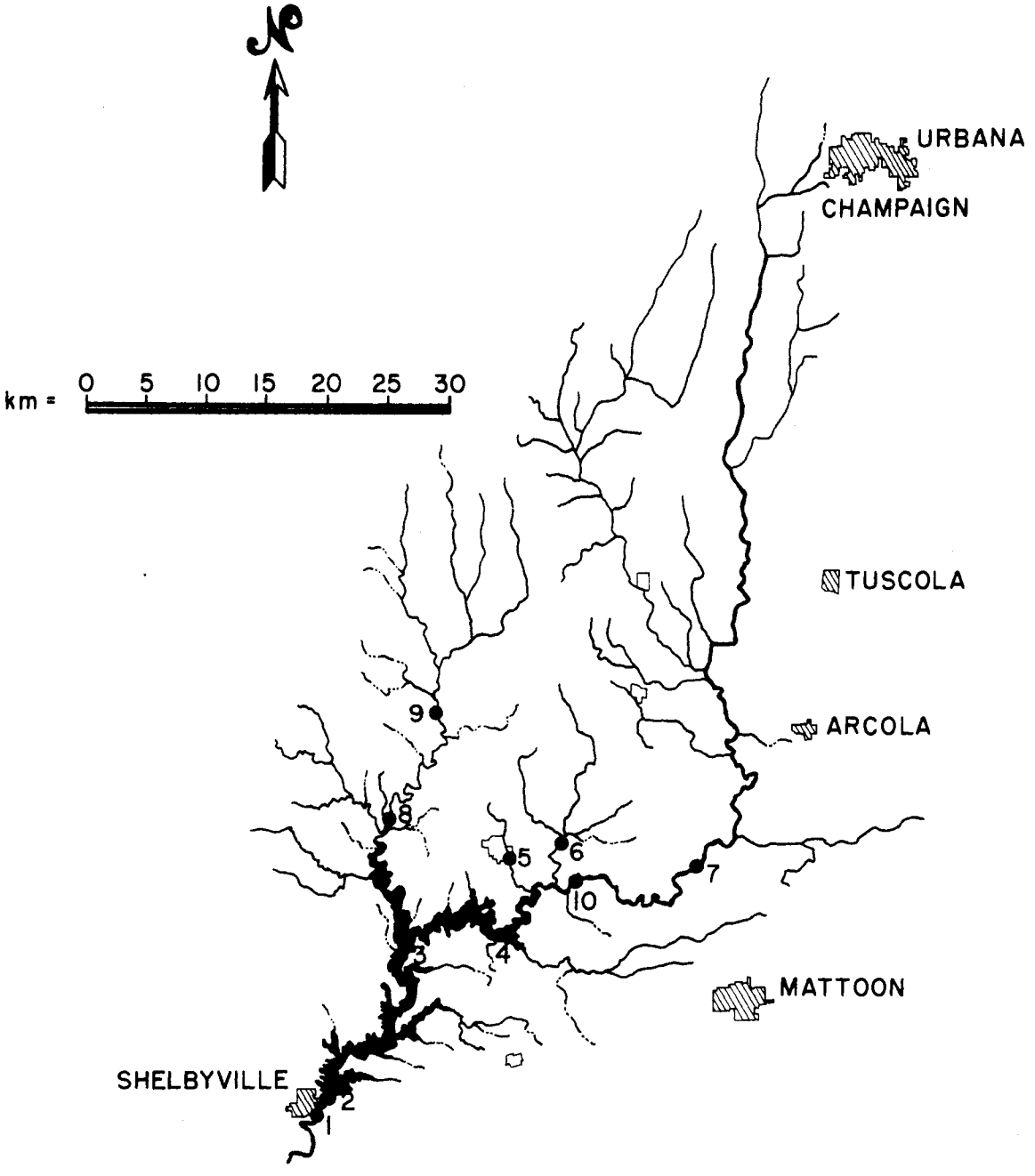

Figure 1. Lake Shelbyville, Kaskaskia River Basin, Illinois, showing the principal collecting sites (Arabic numerals correspond to Roman numerals used in the text).

Iron, Ferrous and Total (mg liter^{-1})

Redox Potential (volts)

Sulfate (mg liter^{-1} as S)

Sulfide, all forms (mg liter^{-1} as S)

Sulfite (mg liter^{-1} as S)

Water Temperature (C)

A series of collections was taken on 6 September 1972, at irregular intervals down the length of the Kaskaskia River from its source in Champaign County through Douglas and Coles Counties to, and including, station X of the regular sampling stations. Each collection in this portion of the study consisted of measurements of the following:

Hydrogen Ion concentration (as pH)

Principal Anions: Bicarbonate (mg liter^{-1}as $CaCO_3$)

Chloride (mg liter^{-1})

Sulfate (mg liter^{-1}as S)

Total Dissolved Ionizable Solids (mg liter^{-1}as NaCl)

Water Temperature (C)

Most water analysis procedures followed in this study are those set forth in the 13th edition of Standard Methods for the Examination of Water and Wastewater (American Public Health Association, American Water Works Association, and Water Pollution Control Federation 1971). Deviations from these methods and the specific method used, where more than one is approved, are listed below.

All analyses were performed in the laboratory unless "field" measurement is stated in the list below. Samples for bacterial pigment analysis were filtered from known volumes of water through a 50 μ glass wool filter and the filter pad frozen until further analysis could be performed. Samples for ferrous iron and sulfite determination were acidified in the field with nitric acid. All other analyses were performed within 1.5 days of collection on unpreserved, raw water samples.

Bacterial Pigments: Acetone extraction method of Takahashi and Ichimura (1968).

Bicarbonate: Potentiometric titration to pH 4.6 (modified from Standard Methods).

Chloride: Specific ion electrode (Beckman, Cedar Grove, New Jersey) and a Century SS pH meter (Beckman).

Dissolved Oxygen: Field measurements using a Clark-type membrane-covered polarographic probe (silver/gold in potassium chloride) (Standard Methods). A Yellow Springs Instruments (Yellow Springs, Ohio) Model 54 dissolved oxygen meter was employed.

Hydrogen Ion Concentration (pH): Field measurements with a Sargent-Welch (Skokie, Illinois) Model PBX meter and glass combination electrode (Standard Methods).

Iron (all forms): Phenanthroline method, with hydroxylamine reduction for total iron (Standard Methods). Optical

density at 510 nm measured with a Bausch and Lomb (Rochester, New York) Spectronic 70 spectrophotometer.

Redox Potential: Field measurements using the platinum electrode method with a Sargent-Welch Model PBX meter.

Sulfate: Turbidimetric method (Standard Methods). Nephelometry employed a Hach (Ames, Iowa) Model 2100 turbidimeter.

Sulfite: Iodometric titration (Standard Methods).

Sulfide (all forms): Field measurements using the methylene blue visual color-matching method for total and dissolved sulfide. Un-ionized hydrogen sulfide by calculation (Standard Methods).

Samples for bacteriological examination were collected on 14 December 1972, from station II in Lake Shelbyville. Samples were taken from 0, 1, 5, 10, 15, and 19m with a Kemmerer sampler. The samples were brought to the Sullivan Laboratory and transferred to airtight containers for transport.

Enrichment and isolation of the green sulfur bacteria generally followed that proposed by Rodina (1972). Briefly, enrichment cultures were established using sediment from the sampling site, calcium sulfate, calcium carbonate, and shredded filter paper as the medium, innoculating with the water sample, providing an anaerobic environment, and illuminating constantly (fluorescent) at 23 C for 3 weeks. Green sulfur bacteria were isolated and

cultured using Larsen's medium (Rodina 1972) and incubated as before.

Good bacterial growth was noted after 2 weeks. Colonies were subjected to acetone extraction and bacterial pigments were determined using the method of Takahashi and Ichimura (1968). Absorption spectra were plotted with a Coleman (Maywood, Illinois) Model 124D spectrophotometer and Sargent-Welch SRG recorder. Replicate colonies were examined microscopically.

The effects of hydrogen sulfide under static anaerobic conditions were determined at 21 C for 24 hours under continuous light conditions. Lake Shelbyville water, filtered through a #25 plankton net, was deoxygenated with a nitrogen-stripping column. Dissolved oxygen was effectively reduced from saturation to 0.2 mg $liter^{-1}$. A stock sulfide solution was prepared by dissolving 1.5 g $Na_2S \cdot 9H_2O$ in 0.1 liters of boiled, glass-distilled water. All sulfide solutions were made using this stock. Dissolved oxygen, pH, and total sulfide were determined for each concentration and the control at 0 and 24 hours. Total sulfide measurements for the bioassay study employed the spectrophotometric method (Standard Methods) using a Bausch and Lomb Spectronic 20 colorimeter.

Triplicate narrow-necked test containers, each holding 500 ml test solution, were used. Five fourth-instar Chaoborus (Diptera:Chaoboridae), recently gathered from Lake Shelbyville,

were introduced into each of two test containers; all containers were stoppered for the duration of the test. The first container was used for 0-hour chemical determinations. At the end of 24 hours, chemical analyses were conducted on one of the two remaining test containers. Observations were made at 0, 4, 8, 12, and 24 hours.

A 195-cm plexiglass column (internal diameter 15 cm), filled with filtered lake water, was utilized in the avoidance study. In experiments I and II, a sulfide solution was prepared by dissolving $Na_2S \cdot 9H_2O$ in water deoxygenated by a nitrogen stripping column. In experiment I the sulfide solution, introduced at the bottom of the water column, occupied the bottom 20 cm of the column, which was filled to a total depth of 130 cm. The Chaoborus, 17 larvae and 13 pupae, were introduced into the column following addition of the sulfide solution. Observations on the distribution of the Chaoborus were made every 15 minutes for 3 hours. Sulfide and pH determinations were made at the end of 3 hours on the water in the column at the bottom (110 to 130 cm), middle, and surface levels.

In experiment II the column was filled with filtered lake water to a height of 110 cm and two 100-watt incandescent bulbs were placed 30 and 40 cm from the surface, respectively, to restrict the movement of the Chaoborus to the lower 90 cm of the

tube. Forty-six Chaoborus larvae were introduced into the column and observed for 1.5 hours. After a 1-hour acclimation period, the distribution of the larvae in the column was recorded. Following the initial distribution observation, a sulfide solution, as described above, was introduced at the bottom of the water column. The sulfide solution occupied the lower 20 cm of the column, which then had a total depth of 130 cm. Observations were made immediately after introduction of the sulfide solution and every hour for 5 hours. Sulfide and pH determinations were conducted as described above.

SOURCES OF SULFUR

In most aerobic freshwater systems, sulfur exists principally in its oxidized form, sulfate. Sulfate is the second most abundant anion, being exceeded only by bicarbonate, in the surface waters of the Midwest.

Six sources are thought to be contributing dissolved sulfur compounds to Lake Shelbyville. These include 1) rain, 2) surface runoff, 3) groundwater 4) decomposition of inundated organic matter, 5) solution of inorganic sulfur compounds from the inundated basin, and 6) pollution. Two of these sources, decomposition of inundated organic matter and solution of inorganic sulfur compounds from the inundated basin, only affect sulfur concentrations within the actual basin of Lake Shelbyville. Further, they represent one-time sources of sulfur which would exist only during the early-impoundment period. The remaining sources, summarized in table 2, contribute to the tributary streams as well and will be discussed below.

The mean annual rainfall in Windsor, Illinois, is 98.68 cm (38.85 inches). For Champaign, Illinois, mean annual rainfall totals 91.16 cm (35.89 inches). Using 95 cm per year as an average rainfall for the total reservoir watershed (2668 km^2, or 1030 $miles^2$). the total rainfall into the Lake Shelbyville basin is approximately 2.53×10^9 m^3. Hutchinson (1957) cites a value of 1.9 mg $liter^{-1}$

Table 2. Sources and sinks of sulfur in the Lake Shelbyville Basin, Illinois, during the period April 1972 through March 1973 (expressed as kg of sulfur).

Sources		Annual Contribution
Rainfall		3.34×10^4
Surface runoff		2.05×10^7
Pollution		4.56×10^6
	Total	2.51×10^7

Sinks		Annual Consumption
Outflow		2.01×10^7
Storage		3.35×10^6
Biological utilization		1.65×10^6
	Total	2.51×10^7

sulfate as an average in North American rain. Using this value, approximately 1.6 x 10^6 kg of sulfur enters the watershed annually as rain during a year with average rainfall. High levels of sulfur dioxide in the air near Tuscola, Illinois, resulting from heavy industry, would make this estimate low. Assimilation of sulfur compounds in the soil, however, account for a portion of this input, so the value 1.6 x 10^6 kg of sulfur per year should be considered a maximum possible contribution from rain during a year of average rainfall.

Rainfall during the period April 1972 through March 1973 was above average; the total being 117.55 cm recorded at the Illinois Natural History Survey's Sullivan Laboratory. Using the calculation method described above, 3.14 x 10^9 m^3 of water containing 1.99 x 10^6 kg of sulfur entered the watershed as rain during the study year. Using the Corps of Engineers' figure of 11,100 acres (4,492 ha) as the mean surface area of the lake, 3.34 x 10^4 kg of sulfur (as sulfate) fell directly into the lake and would not be accounted for in measurements of tributary streams.

In calculating the sulfur contribution of surface runoff to Lake Shelbyville, measurements from the portion of the Kaskaskia River between U. S. Highway 36 west of Tuscola, Douglas County, and the upper portion of Lake Shelbyville must be omitted. This is because of industrial pollution in the Tuscola area producing high concentrations of sulfate downstream from that point. This

sulfur input will be discussed in detail below.

Measurements of sulfate in streams tributary to Lake Shelbyville (Table 1, stations V, VI, VIII, IX) yield mean concentrations from 13.5 mg liter^{-1} as S, in Jonathan Creek, to 18.7 mg liter^{-1} as S, in Asa Creek. The Illinois Environmental Protection Agency (1971) reports a range of sulfate from 1.7 to 308 mg liter^{-1} as S (mean equals 19.7) from 183 measurements taken in the Kaskaskia River 6 km upstream from the industrial outfall near Tuscola. The weighted mean for all of these surface water sources equals 18.5 mg liter^{-1} as S. This sulfur is derived from rain and from solution of soluble sulfur compounds in and on the soil.

No direct measurements were made of the total volume of surface water entering Lake Shelbyville during the study year. It is possible, however, to calculate this value.

Discharge data supplied by the Corps of Engineers for Shelbyville dam yield a mean value of 29.93 m^3 sec^{-1}, thus 9.44 x 10^8 m^3 of water passed through the dam during the period of study.

Water was also lost from Lake Shelbyville through evaporation. Published data for lake evaporation in Illinois (Roberts and Stall 1967) give an approximate value for annual evaporation in the Lake Shelbyville area of 35 inches (89 cm). Using the Corps of Engineers' figure of 11,100 acres (4,492 ha) as the mean surface area of Lake Shelbyville, evaporative water loss for the study year amounted to 3.99 x 10^7 m^3.

Water level in Lake Shelbyville made a net increase of 4.18 m from 6 April 1972 to 28 March 1973. This represented an increase in storage of 1.72×10^8 m^3.

Discharge through the dam, evaporation, and increase in storage, summed, equal the total volume of water intering Lake Shelbyville. This amounts to 1.16×10^9 m^3 of water for the study year. Subtracting rainfall directly into the reservoir (117.55 cm over 4,492 ha equals 5.28×10^7 m^3) leaves 1.11×10^9 m^3 of water entering the lake from other sources, namely groundwater and surface runoff. Groundwater will be discussed further below, but is not considered as a source of water during the study year. Hence, it is calculated that 1.11×10^9 m^3 of water entered Lake Shelbyville as surface water during the study year. Combining this volume with the 18.5 mg $liter^{-1}$ of sulfur for unpolluted tributary streams yields a sulfur input of 2.05×10^7 kg from surface runoff.

Groundwater as a source of sink of sulfur has not been included in the calculations represented here. The direction of flow is determined by the height of the water table relative to the height of the surface of the reservoir. Flow would be from the higher level to the lower level. During the time span of this study (April 1972 through March 1973), Lake Shelbyville had a net rise in water level of 4.18 m. Therefore, it has been assumed that during this study net flow was from the lake into the ground-

water. With the mean sulfur concentration in the lake water being 17.2 mg liter^{-1}, 1 kg of sulfur would have left the lake with every 58 m^3 of water which infiltrated the groundwater. It was not within the scope of the present study to measure interchange of water between the reservoir and the groundwater, therefore sulfur losses via this mechanism cannot be estimated.

In a long-term study which includes both high and low water periods in Lake Shelbyville, exchange of water between the lake and the groundwater should approach equilibrium. Mean groundwater concentrations of sulfur from transects along the Sangamon River, Macon and Piatt Counties, Illinois, vary from 10.1 to 18.1 mg liter^{-1}. These values are very close to the mean sulfur concentration in Lake Shelbyville (17.2 mg liter^{-1}). From this it may be concluded that sulfur interchange between groundwater and the lake also approaches equilibrium.

The principal source of pollution by sulfur in the Lake Shelbyville watershed is an industrial complex west of Tuscola, Illinois. Phosphoric acid is manufactured from sulfuric acid and calcium phosphate through the reaction

$$3\ H_2SO_4 + Ca_3(PO_4)_2 = 2\ H_3PO_4 + 3\ CaSO_4$$

Calcium sulfate is a by-product of this process and is stored in a large dump pile adjacent to the Kaskaskia River. Erosion from this dump carries calcium sulfate directly into the river. In her study of the limnology of Asa Creek and the Kaskaskia River,

Brigham (1972) demonstrated significant correlations (0.001 level) between calcium and sulfate concentrations at her river stations. Further, she found that stations in the Kaskaskia River yielded significantly higher sulfate concentrations (0.05 level) than stations in Asa Creek. This supports the premise that calcium sulfate is the principal form of sulfur present in the Kaskaskia River and that its source is the industrial complex near Tuscola, Illinois.

The Illinois Environmental Protection Agency (1971) reported a mean sulfate concentration of 59 mg liter^{-1} (19.7 mg liter^{-1} as S) from 183 measurements taken 4 miles (6.3 km upstream of the industry). They reported a mean concentration of 291 mg liter^{-1} (97 mg liter^{-1} as S) from 126 measurements immediately downstream from the industry. The mean increase in sulfur from these measurements is 77.3 mg liter^{-1} as S.

The U. S. Geological Survey operates gaging stations 17 km upstream and 32 km downstream from the Tuscola complex. Interpolation from their mean flow data (U. S. Geological Survey 1971) for these stations yields a mean flow of 1.87 m^3 sec^{-1} for the Kaskaskia at the site of the calcium sulfate contamination. Combining this flow with the mean increase in sulfur from the industry calculated above (77.3 mg liter^{-1}) yields a daily sulfur loading of 1.25×10^4 kg. If it is assumed that all of this sulfur enters the stream as calcium sulfate, 5.48×10^4 kg of calcium sulfate enter the river each day. The annual contribution of sulfur from this source to Lake Shelbyville is approximately 4.56×10^6 kg.

FLOW-CONCENTRATION RELATIONSHIPS

Observed relationships between flow and sulfur loading at stations I and X are shown in figures 2 and 3, respectively. These lines were generated from the raw data using least-squares regression analysis. A t-test of the data showed both lines to be significant (0.001 level). In order that these lines might be used to predict accurately sulfate loading from discharge measurements, confidence belts (0.001 level) were calculated and plotted along with their respective regression lines. In both cases, the confidence interval was narrow. Table 3 contains estimates of sulfate concentration, with range of error of estimation, for the maximum, minimum, and mean discharges measured at stations I and X.

The regression lines in figures 2 and 3 are not parallel. They intersect at a point corresponding to a discharge of 0.57 $m^3\ sec^{-1}$. At this discharge, sulfur flowing into the lake equals sulfur flowing out through the dam. With discharges in and out of the lake below this value, Lake Shelbyville accumulates sulfur. With discharges above this value, the lake is stripped of accumulated sulfur.

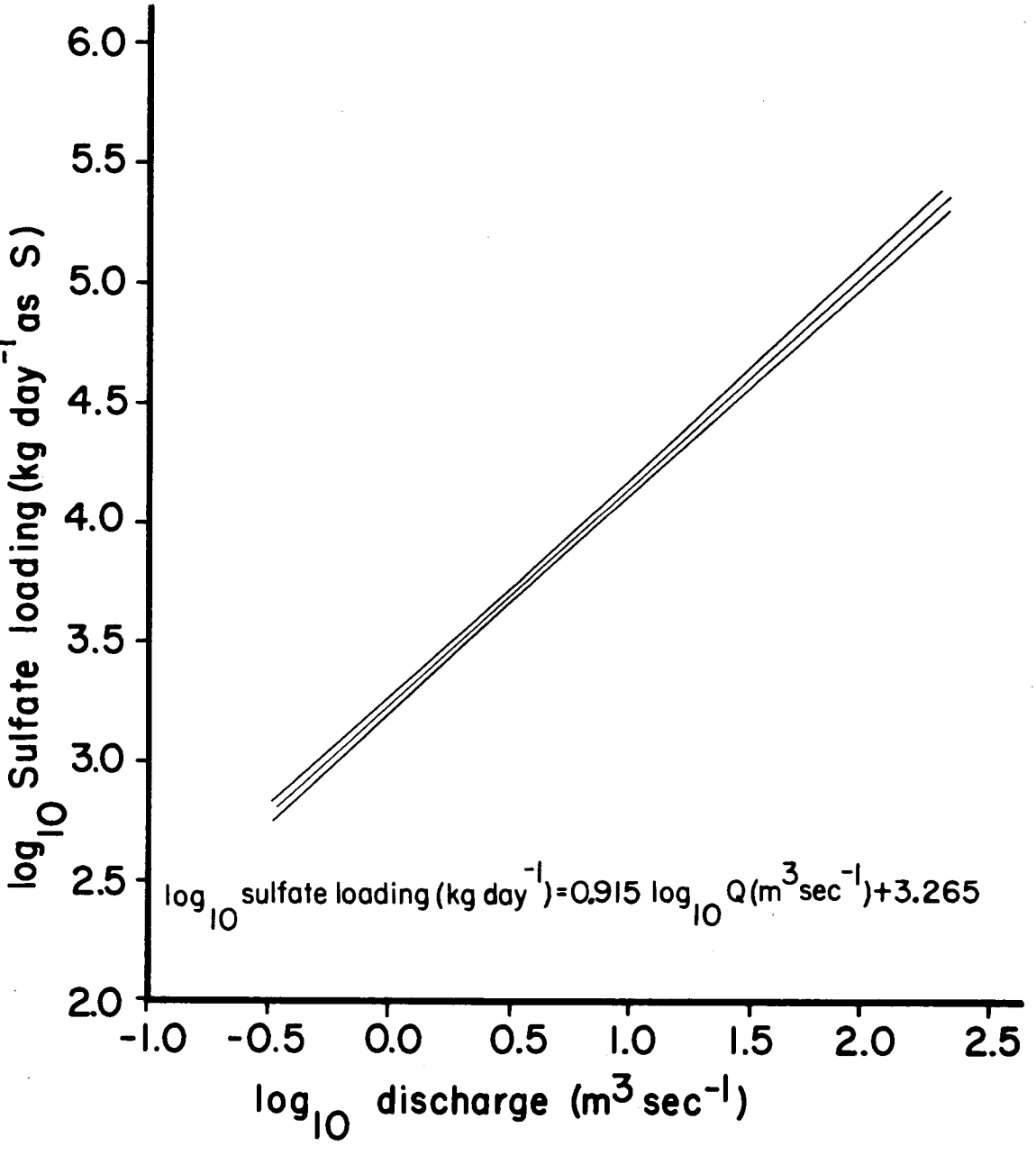

Figure 2. Least squares regression of sulfate loading and discharge measurements, with confidence belts (0.001 level), derived from 58 paired measurements from Station I, Lake Shelbyville, Illinois, during the period 24 April 1970 through 28 March 1973.

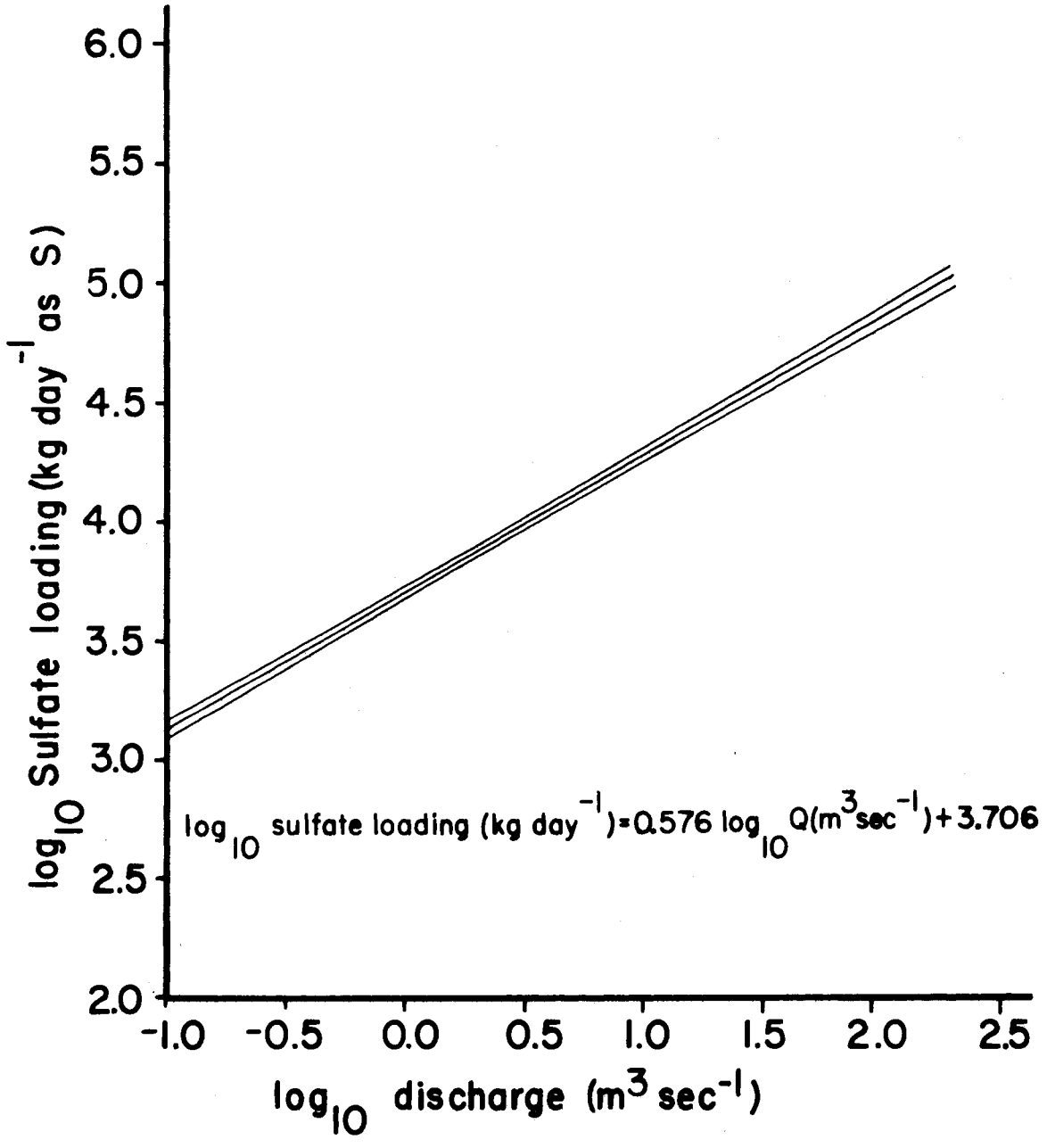

Figure 3. Least squares regression of sulfate loading and discharge measurements, with confidence belts (0.001 level), derived from 160 paired measurements from station X, Lake Shelbyville, Illinois, during the period 19 July 1971 through 21 August 1972.

Table 3. Estimates of sulfate concentration from discharge measurements, with error estimate (0.001), for two stations in the Lake Shelbyville Basin, Illinois.

Station	Discharge[1] (m^3 sec^{-1})		Sulfate mg $liter^{-1}$ as S)	Range of Confidence	% Possible Error
I	Q_{max}	85.5	14.6	1.7	11.6
	Q_{min}	0.28	23.7	1.3	5.5
	Q_{mean}	2.55	19.7	1.8	9.1
X	Q_{max}	82.2	8.4	0.4	4.8
	Q_{min}	0.54	90.2	4.1	4.5
	Q_{mean}	5.24	30.8	0.6	1.9

[1] The discharge measurements recorded here may not agree with values reported elsewhere in this paper. Values presented here were drawn from all data where paired measurements of both discharge and sulfate were taken.

SULFUR BALANCE

The three sources of sulfur discussed above (Table 2) represent an input of 2.51×10^7 kg of sulfur to Lake Shelbyville from April 1972 through March 1973. A substantial portion of this sulfur passed out through Shelbyville Dam in the discharge water. As stated above, 9.44×10^8 m^3 of water passed through the dam during the study year. The mean rate of discharge was, therefore, 29.93 m^3 sec^{-1}. Using the equation for station I calculated above (Fig. 2), the mean daily outflow of sulfur through the dam was 5.51×10^4 kg, or 2.01×10^7 kg $year^{-1}$.

Storage increased by 1.72×10^8 m^3 from April 1972 through March 1973. During this period the mean sulfate concentration of Lake Shelbyville decreased from 20.9 mg $liter^{-1}$ to 18.6 mg $liter^{-1}$ (both as S). The total sulfur content of the lake, therefore, increased by 3.35×10^6 kg.

There remains 1.65×10^6 kg of sulfur to be accounted for. Possible sulfur sinks include infiltration to the groundwater, conversion to sulfur forms other than sulfate, and utilization by the biota.

As pointed out above, there was a net rise of 4.18 m in reservoir level during the study year. It is therefore assumed that water from Lake Shelbyville flowed from the lake into the groundwater. No measure of this flow was possible, hence the

amount of sulfur leaving the lake via this route is unknown. This loss may represent a substantial portion of the sulfur which otherwise has not been accounted for.

It is doubtful that the unaccounted sulfur changed from sulfate to some other inorganic ion. Sulfide analyses throughout the study never detected this form of sulfur. Sulfite concentrations (Table 1) did not vary appreciably during the study.

Autotrophic organisms and a number of heterotrophs utilize sulfur in the form of sulfates in the manufacturing of some amino acids (Dussart 1966). The rate of utilization by these organisms was not measured in the present study.

If it is assumed, however, that all of the inflowing sulfur which did not flow out through the dam, 1.65×10^6 kg, was incorporated by the biota, it should be possible to calculate, approximately, a maximum rate of production of organic matter for the lake. Using an approximation of 0.8% for the sulfur content of organic matter, 1.65×10^6 kg of sulfur could result in the production of 2.06×10^8 kg of organic matter. With the carbon content of organic matter approximately 50% (Kuznetsov, Romanenko, and Karpova 1969), carbon fixation for Lake Shelbyville during the study year would amount to 1.03×10^8 kg. Dividing this value by the mean surface area of Lake Shelbyville yields a maximum carbon fixation rate of approximately 2 kg m^{-2} $year^{-1}$. This value is

higher than the values reported by Kuznetsov, Romanenko and Karpova (1969), who utilized similar calculations for Rybinsk Reservoir in the U.S.S.R. The difference may represent the sulfur loss to the groundwater not considered in the present study.

ANNUAL SULFUR CYCLE

Sulfate concentrations in Lake Shelbyville were generally lower during the winter months than during the remainder of the year (Fig. 4). Sulfate concentrations reflected homogeneity when the lake was not stratified thermally.

Mortimer (1941 , 1942) found that sulfate declined in deep water over mud in Esthwaite Water during summer stratification while high spring values persisted at the surface. This decrease above the mud-water interface is to be expected when the redox potential of the mud surface falls low enough. Figure 4 frequently shows this decline in sulfate concentration above the sediments during the period of thermal stratification. A single series of redox potential measurements of sediments at station II on 28 July 1972 yielded a mean value of -0.15 volts.

Mortimer concluded that the reduction of sulfate occurred almost exclusively at the mud-water interface and not in the free water. He did not detect hydrogen sulfide chemically in any of the Esthwaite Water samples. He observed the greatest decline in sulfate during mid-August, when ferrous iron reached a maximum and dissolved oxygen had entirely disappeared. Reduction of sulfate and the formation of ferrous sulfide, relatively insoluble at the pH values he encountered (6.7), would explain the lack of sulfides in the water.

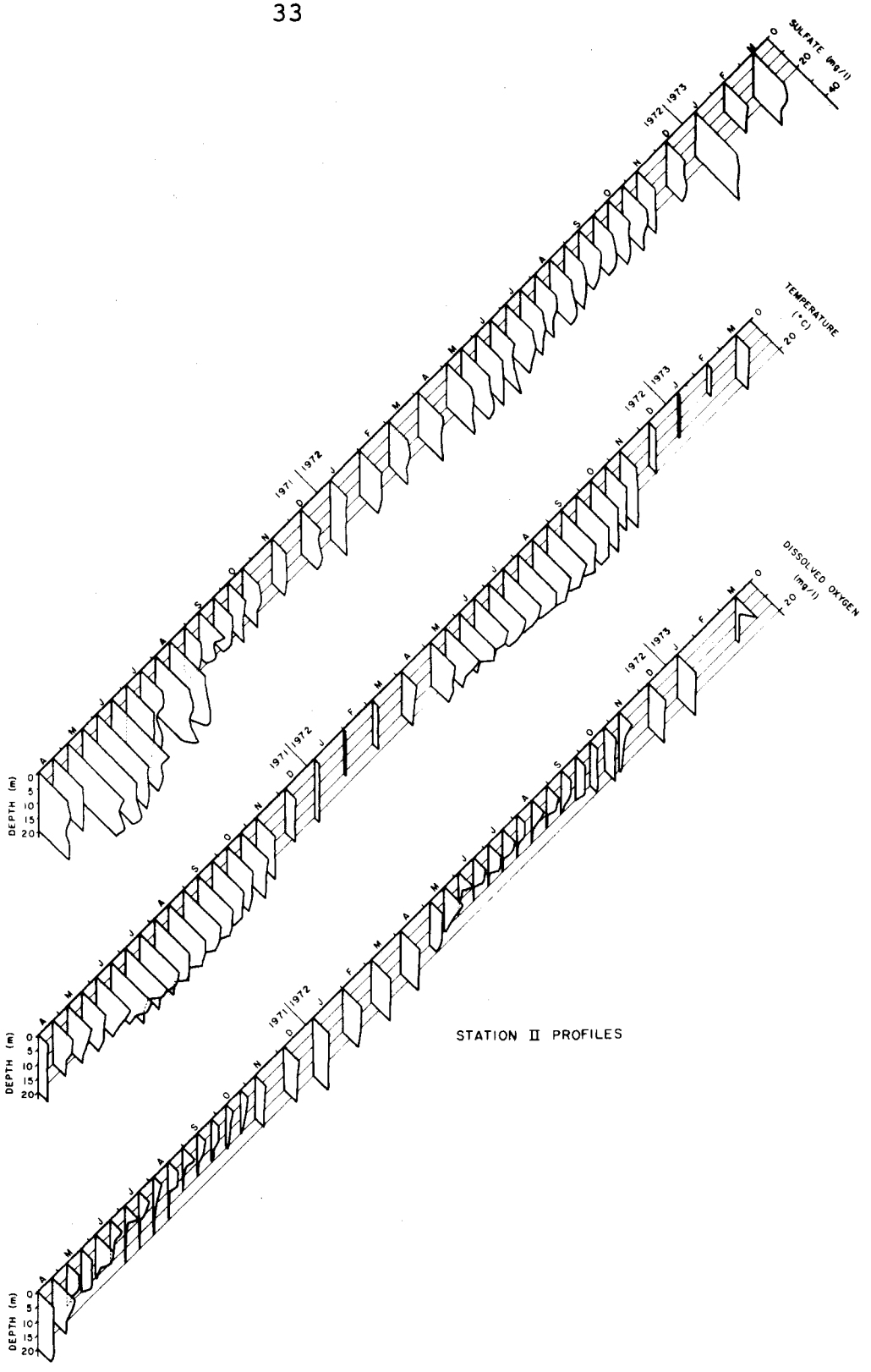

Figure 4. Sulfate (as S), water temperature, and dissolved oxygen profiles from station II, Lake Shelbyville, Illinois, for the period April, 1971, through March, 1973.

Substantial amounts of ferrous iron were present in Lake Shelbyville during this study (Table 1). Further, pH values were relatively high. From this it is logical to conclude that the sulfate reduction observed over the sediments was accompanied by the production of relatively insoluble ferrous sulfide, hence dissolved sulfides would not be liberated into the water from this process.

In addition to this chemical reduction, a second type of conversion of sulfate to sulfide, by sulfate-reducing bacteria, also occurs. These organisms are anaerobic heterotrophs which utilize sulfate as the terminal electron acceptor in metabolic oxidation and release hydrogen sulfide (Gooch 1968). It is nearly always possible to find evidence of such reduction wherever sulfate and organic matter coexist in the absence of oxygen and ferrous iron (Hutchinson 1957). Kriss (1961) reported that 99.5% of hypolimnetic hydrogen sulfide was produced by the bacterial reduction of sulfates.

Several investigators have studied conditions prior to and during hydrogen sulfide production. Takahashi and Ichimura (1968) reported hydrogen sulfide in the hypolimnion of a meromictic lake throughout the year, while in other lakes they studied, it was only detected during summer stratification. During this period they found hydrogen sulfide concentrations from 0.5 to 1.6 mg liter^{-1} (as S) in freshwater lakes where no oxygen was

detected and the pH was 7.0. Lighthart (1963) reported sulfide concentrations of 0.2 to 1.1 mg liter^{-1} (as S) in San Vincente Reservoir. He detected no hydrogen sulfide until the supply of dissolved oxygen was exhausted and the redox potential of the sediment approached -0.09 v. At this time he also found an increase in the number of sulfate-reducing bacteria. The sulfate concentrations 1 m above the sediment and the pH of the sediment ranged from 86 to 90 mg liter^{-1} (as S) and 6.9 to 7.5, respectively.

Summer, 1971 was the first summer of impoundment for Lake Shelbyville. Large amounts of organic matter were present within the basin inundated by the filling of the lake. The sulfate concentration of the lake was high (Fig. 4). Deoxygenation of the hypolimnion began as early as May, and by July, the hypolimnion was essentially anoxic.

Sulfate-reducing bacteria liberated detectable quantities of hydrogen sulfide during July, August, and September, 1971. It is believed that the decline in sulfate shown during July, 1971 (Fig. 4), represents sulfur converted to the sulfide form by sulfate-reducing bacteria.

During late July and August, 1971, midwater samples from Lake Shelbyville were distinctly violet in color. Although no microbiological investigations were undertaken at that time, the nature of the color and its distribution in the lake strongly suggest the presence of the so-called purple sulfur bacteria

(Thiorhodaceae) (Hutchinson 1957). These organisms use hydrogen sulfide as an oxygen acceptor in the photosynthetic reduction of carbon dioxide. The purple sulfur bacteria can carry the oxidation of hydrogen sulfide as far as sulfate. The rise in sulfate concentration during August, 1971, is attributed to these organisms.

During the period of this study, April, 1972 through March 1973, hydrogen sulfide was not detected in Lake Shelbyville and the presence of purple sulfur bacteria was not indicated. The latter is not surprising, as purple sulfur bacteria are generally associated with high concentrations of hydrogen sulfide (Kondrat'eva 1965). From this it follows that either bacterial reduction of sulfate to hydrogen sulfide did not take place during the study year or that the rate of reduction was low and that near-simultaneous oxidation by sulfide-oxidizing bacteria precluded the accumulation of detectable sulfide concentrations.

Green sulfur bacteria (Chlorobacteriaceae) are the predominant sulfur-oxidizing bacteria when concentrations of hydrogen sulfide are low (Kondrat'eva 1965). Microbiological investigations, therefore, were directed toward this group. Pigment-analysis techniques demonstrated the presence of Bacterioviridine -654 and Bacterioviridine -662 throughout the study period (Table 1). These pigments are known to be present in green sulfur bacteria. An additional pigment, Bacteriochlorophyll -772, was also present throughout the study period.

The presence of green sulfur bacteria, suggested by the pigment-analysis tests, was confirmed by growth of bacteria on media selective for green sulfur bacteria, and by microscopic examination of smears from enrichment cultures of Lake Shelbyville samples (Fig. 5). It is likely that the particular organism present in the lake was a species of Chlorobium.

The great amount of newly inundated organic matter available to sulfate-reducing bacteria during summer, 1971 was not present during summer 1972. Sulfate concentrations were also lower during 1972 (Fig. 4). Sulfate reduction under these conditions would not proceed at the high rate of the previous year. The presence of green sulfur bacteria during 1972 indicates that some sulfate reduction was taking place, but the lack of detectable hydrogen sulfide indicates an equilibrium between the sulfate-reducing bacteria and the sulfide-oxidizing green sulfur bacteria.

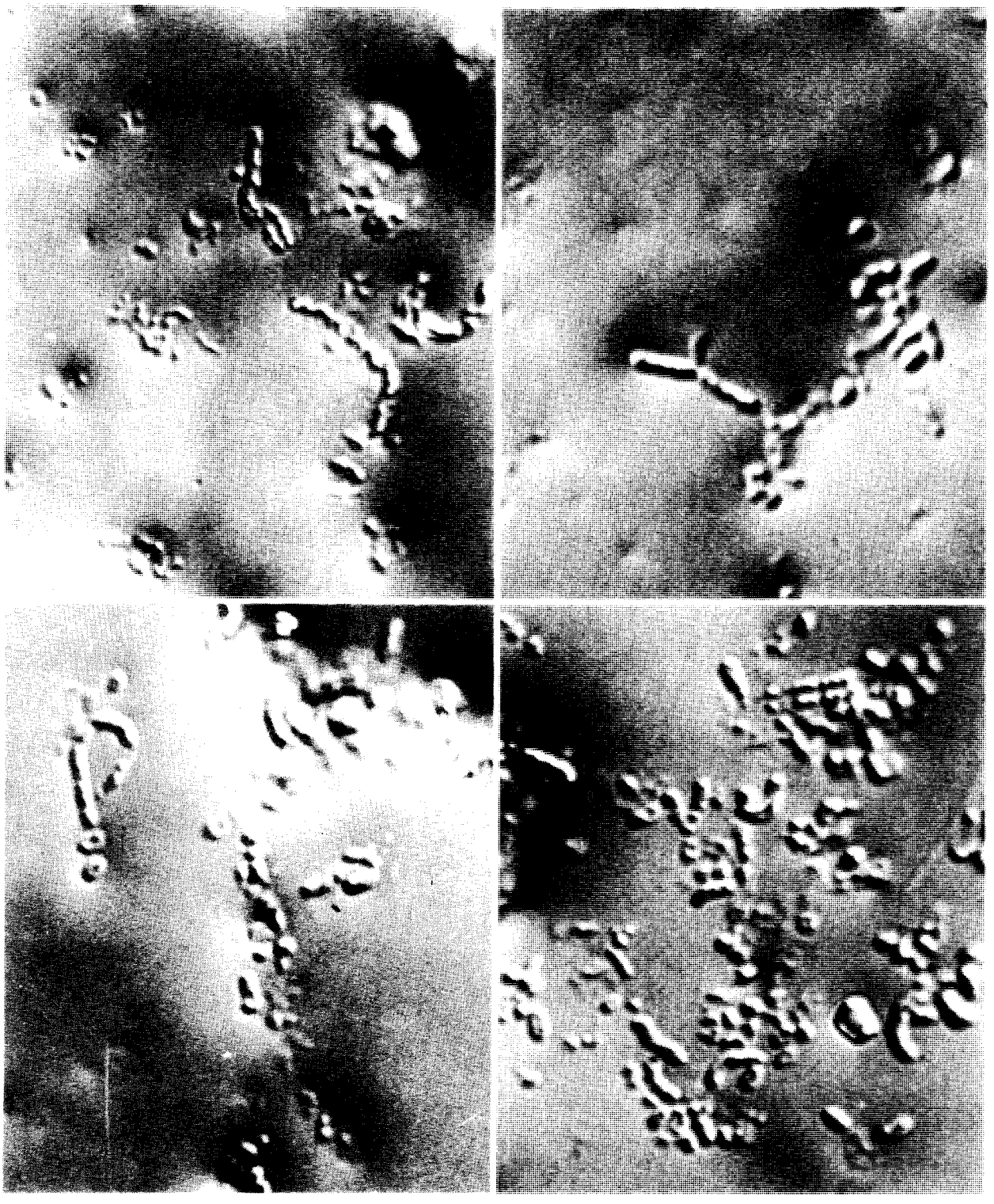

Figure 5. Photomicrographs of green sulfur bacteria from enrichment cultures of water from the 19 m depth at station II, Lake Shelbyville, Illinois.

EFFECTS OF SULFIDES UPON AQUATIC LIFE

The importance of hydrogen sulfide to aquatic life frequently has been overlooked because it is rapidly removed from the water and because of the difficulty in sampling the water directly above the mud-water interface. In this restricted area both fish-food organisms and the eggs and fry of many fishes occur, and exposure to hydrogen sulfide might influence the fauna significantly (Smith and Oseid 1972).

Sulfide may exist in one of three forms H_2S, HS^-, or $S^=$; the proportion of each depending primarily upon the pH value. Only at pH 10 and above is the sulfide ion ($S^=$) present in appreciable proportions. At pH 7, HS^- and H_2S are present in nearly equal abundance. At lower pH values, H_2S predominates. Thus, when reference is made to sulfides in water, the sulfide is probably in the form of HS^- or H_2S (McKee and Wolf 1963). The toxicity of sulfide solutions increases as the pH value is lowered, i.e. H_2S appears to be the most toxic form rather than the ions HS^- or $S^=$.

A number of studies concerning toxicity of hydrogen sulfide to aquatic organisms and fish have proposed safe levels. Haydu, et al. (1953) determined the maximum concentration of hydrogen sulfide tolerated without injury by the most sensitive fish tested

as 0.3 mg liter^{-1}, while Nellist (in McKee and Wolf 1963) considered 1.0 mg liter^{-1} to be the limiting toxic concentration. Murdock (in McKee and Wolf 1963) set the critical concentration at 0.5 to 1.0 mg liter^{-1}.

Colby and Smith (1967) showed that hydrogen sulfide levels of 0.3 mg liter^{-1}, under controlled conditions approximating river conditions, were acutely lethal to Gammarus pseudolimnaeus and Stizostedion eggs and fry. Gammarus pseudolimnaeus was intolerant to low dissolved sulfide concentrations (0.16 to 0.36 mg liter^{-1}) especially at low dissolved oxygen concentrations (1.2 to 1.3 mg liter^{-1}). When the oxygen concentration was higher (5.0 to 5.1 mg liter^{-1}), G. pseudolimnaeus was much more tolerant of sulfide. Dissolved sulfide at concentrations of 0.33 mg liter^{-1} and above were highly toxic to Stizostedion eggs and newly hatched fry, and concentrations of 0.14 and 0.23 mg liter^{-1} had an appreciable effect on survival. Sac-fry were more vulnerable to dissolved sulfide than were the eggs.

Smith and Oseid (1972) reported TL_{50} values for various fish eggs at 4 to 10 days from 0.022 to 0.064 mg liter^{-1} hydrogen sulfide under different conditions of oxygen and temperature and TL_{50} for Stizostedion fry at 0.007 mg liter^{-1}. The proposed safe level was less than 0.006 mg liter^{-1}.

There are few toxicity results as sensitive as that reported by Colby and Smith (1967) and Smith and Oseid (1972) for aquatic

invertebrates other than Gammarus. Van Horn, et al. (1949) reported a minimum lethal concentration of hydrogen sulfide for Daphnia and Ephemeroptera nymphs as 1.0 mg liter^{-1}, and for chironomid larvae as 750 mg liter^{-1}.

ECOLOGY OF *CHAOBORUS*

Chaoborus, the predominant benthic organism during summer in Lake Shelbyville, is an important constituent of both the benthic and planktonic communities. Juday (1921) reported that *Chaoborus* larvae descend from the limnetic to the benthic habitat during the first 7 to 10 days of development. The larvae, primarily first instar, spend at least 1 week continually in the limnetic community. Eggleton (1932) found that increase in size of larvae corresponded with increased depth distribution. The extreme range of the limnetic larvae in terms of size-age groups (2.45 to 7.65 mm) showed them to be ranging from very young larvae to those nearly three-fourths grown. This meant that the majority of the limnetic population ranged up to half-grown size. Stahl (1966) found second instar larvae to occur only in the plankton. About 25 to 50% of the third instars and 95 to 100% of the fourth instar were benthic.

The vertical migratory movements of *Chaoborus* are well-known (Juday 1921; Eggleton 1932; Wood 1956) and involve an ascent of the benthic larvae (predominantly fourth instar) into the limnetic zone about dusk, and a descent beginning in the early hours the following morning. Teraguchi and Northcote (1966) described the migrating larvae as follows: first instar larvae remained in the epilimnion and exhibited no migration; second instar larvae, vertically distributed as the first instar, showed a weak migration

to the surface at night; third instar larvae had a deeper depth distribution and underwent a stronger vertical migration than the second instar larvae; and the fourth instar larvae, present in the hypolimnion during the day, demonstrated a marked migration to the surface at night. The fourth instar larvae have been reported frequently as inhabiting the benthic community when the hypolimnion is anaerobic and hydrogen sulfide is present (Hunt 1958; Teraguchi and Northcote 1966).

Although Chaoborus is known for its ability to exist in anaerobic conditions (Eggleton 1932), the length of exposure to the anaerobic and hydrogen sulfide conditions would usually not exceed 12 hours, due to the vertical migration of the benthic Chaoborus to the surface at night.

No toxicity literature exists for the effects of hydrogen sulfide on Chaoborus. This study was initiated to determine a minimum effect level of hydrogen sulfide under anaerobic conditions to the fourth instar larvae of Chaoborus. An effort was also made to determine whether the fourth instar larvae and pupae of Chaoborus activity would avoid high hydrogen sulfide concentrations by migrating vertically out of the hydrogen sulfide environment.

STATIC BIOASSAY

The survival results of the static sulfide test after 4 hours of exposure were 100% for the control, 90% for 0.25 and 0.5 mg liter^{-1} as S, and 0% for all other concentrations. After 24 hours, only 10% of the Chaoborus survived at the control and 0.25 and 0.50 mg liter^{-1} as S. All organisms at concentrations 1.0, 5.0, and 10.0 mg liter^{-1} as S went to the top of the test containers within 4 hours. This appeared to be either a reaction to stress or a result of death. At the end of 24 hours, all larvae in all test containers were at the top of the containers; all were dead except three. Observations at 8 and 12 hours were difficult to evaluate because distinction between total immobilization and death was difficult. Berg, et al. (1962) reported that activity of Chaoborus was independent of oxygen supply. Therefore, the sulfide rather than the low oxygen tension may account for the total immobilization observed at 4 hours at concentrations 1.0 mg liter^{-1} as S and higher.

Extreme care and haste were exercised in preparing each concentration for the static test. However, dissolved oxygen increased during preparation from 0.2 to around 0.8 mg liter^{-1}. During the test dissolved oxygen only increased 0.1 mg liter^{-1} in each concentration (Table 4). The level of sulfide did change considerably because of the greater pH fluctuation.

Table 4. Chemical parameters measured during the 24-hour static bioassay at 0 and 24 hours.

Nominal Sulfide Concentration (mg liter^{-1}as S)	Hour	Dissolved Oxygen(mg liter^{-1})	pH	Total Sulfide (mg liter^{-1}as S)
10.0	0	0.7	9.4	10.0
	24	0.8	9.2	7.7
5.0	0	0.9	8.9	4.5
	24	1.0	8.8	1.8
1.0	0	0.8	8.6	0.64
	24	0.9	8.2	0.32
0.5	0	0.9	8.5	0.48
	24	1.0	7.8	0.24
0.25	0	1.2	8.3	0.32
	24	1.1	7.5	0.24
Control	0	0.9	8.6	----
	24	1.0	7.7	----

Death resulted within 4 hours at a measured sulfide concentration of from 0.64 to 0.32 mg liter^{-1} as S. Therefore, the minimum level at which an effect was observed within 4 hours was 0.64 mg liter^{-1} as S for Chaoborus. This value is much more sensitive than the 750 mg liter^{-1} value reported by Van Horn, et al. (1949) for chironomid midges, and probably represents a more realistic acute toxic value for benthic organisms.

AVOIDANCE EXPERIMENTS

The results of Experiment I (Tables 5 and 6) show a definite avoidance by both the Chaoborus pupae and larvae of the anaerobic water containing 0.8 mg liter^{-1} as S of sulfide. All of the pupae (Table 5) went to the bottom of the water column when introduced. Only 8% of these failed to enter the sulfide stratum (110-130 cm depth). Within 60 minutes, however, only 46% of the pupae remained in the sulfide stratum. After 75 minutes all pupae remaining in the sulfide stratum (23%) were dead. Throughout this experiment, pupae migrated to the upper 30 cm of the column.

The pupae responded more rapidly to the sulfide stratum than did the larvae (Table 6). After 60 minutes, approximately the same percentage of larvae remained in the sulfide stratum as when the larvae were introduced into the column. After 75 minutes the larvae in the sulfide stratum (14%) were dead; all others had migrated out of the bottom stratum. A few larvae later re-entered the sulfide stratum, and, after 180 minutes, all larvae observed in the sulfide stratum (20%) were dead.

In Experiment II (Table 7) only Chaoborus larvae were introduced into the water column. They were allowed to acclimate for 90 minutes prior to the introduction of the sulfide water (1.7 mg liter^{-1} as S) at the bottom. The lights apparently restricted all larvae to the lower 100 cm stratum. Initially, 33% of the larvae

Table 5. Distribution expressed as percentage of Chaoborus pupae after introduction into a stratified water column.

Depth in Column	Sulfide (mg liter^{-1} as S)	(Time - (minutes) 0	15	30	45	60	75	90	180
0 to 30 cm	0	--	8	23	23	39	54	69	69
30 to 90 cm	0	--	15	8	8	--	--	--	--
90 to 110 cm	0	8	--	15	8	15	23	8	--
110 to 130 cm	0.8	92	77	54	61	46	23*	23*	31*

* Dead

Table 6. Distribution expressed as percentage of Chaoborus larvae after introduction into a stratified water column.

Depth in Column	Sulfide (mg liter^{-1} as S)	Time - (minutes) 0	15	30	45	60	75	90	180
0 to 30 cm	0	--	6	12	12	6	7	25	27
30 to 90 cm	0	35	6	--	6	19	36	25	20
90 to 110 cm	0	18	41	44	29	31	43	19	33
110 to 130 cm	0.8	47	47	44	53	44	14*	31**	20*

*Dead

**Includes those larvae recorded as dead at 75 minutes.

Table 7. Distribution expressed as percentage of Chaoborus larvae before and after introduction of anaerobic sulfide water.

Depth in Column	Sulfide (mg liter^{-1} as S)	Time - (hours)				
		Before S	0* After S	1	2	5
0 to 40 cm	0	--	--	--	--	--
40 to 90 cm	0	58	60	65	42	57
90 to 110 cm	0	9	32	27	52	37
110 to 130 cm	1.7	33	8	8	6	6

*Counts made immediately prior to and after the addition of the sulfide solution to the bottom 20 cm of the column.

were located in the bottom stratum. Upon introduction of the sulfide, the larvae immediately moved to the water layer adjacent to the sulfide stratum; only 8% of the larvae remained in the sulfide stratum. After 5 hours the distribution was similar to the distribution occurring when sulfide was introduced into the bottom stratum. All larvae were alive at the end of the test.

The mortalities (20% of the larvae and 31% of the pupae) resulted in Experiment I when the animals had to acclimate themselves in the water column which initially contained an anaerobic sulfide bottom stratum. The animals seemed to orient from the bottom; that is, almost all of the animals (larvae and pupae) went to the bottom first, then moved up in the column. This forced exposure upon the animals before they were allowed to acclimate within the column. As a result, 14% of the larvae and 23% of the pupae in Experiment I never moved out of the sulfide stratum. Also, a few which were able to leave the lower stratum apparently never recovered from exposure to sulfide. This accounts for the mortalities resulting in Experiment I. In Experiment II the larvae were acclimated within the column prior to introduction of the sulfide. Although 6% of the larvae remained at the bottom, no mortalities resulted. There was probably movement both ways between the 90 to 110 cm and the 110 to 130 cm strata, and the 6% of the larvae could involve different larvae moving between the two strata.

RECOMMENDATIONS AND IMPORTANCE TO WATER RESOURCES RESEARCH

Data gathered prior to and during this study support the premis that hydrogen sulfide production can be expected during the early period of reservoir impoundment. Conditions favoring sulfide production include a moderate sulfate concentration, a large amount of inundated organic matter, and an anaerobic hypolimnion.

The operation plan for Lake Shelbyville called for drawing discharge water from the hypolimnion during summer. With hydrogen sulfide present, this resulted in a substantial air pollution problem in the vicinity of the dam. By altering discharge to include only water from the epilimnion, sulfide odors in the tailwater area were eliminated. It is recommended that provision for both epilimnion and hypolimnion outflow be incorporated into dam design. Substantial water quality differences may exist between the epilimnion and hypolimnion of a reservoir. With two alternatives for discharge water the reservoir manager is in a better position to optimize water quality within a reservoir system.

The relationship between sulfate loading and discharge may prove useful to the limnologist and water-resource manager alike. Water chemistry records in the Midwest are generally available only for recent years. Discharge data, however, have been gathered by the U. S. Geological Survey for long periods at a large number of

sites. Once a loading-versus-discharge relationship has been established in a stream, it may be possible to project approximate loading data for the entire period of discharge record. Similar relationships between loading of other substances versus discharge may prove to be a useful tool to the limnologist.

For the water-resource manager, regression analyses for stations below and above an impoundment, such as those in figures 2 and 3, respectively, make it possible to calculate whether or not sulfur is accumulating in a reservoir. If production of sulfide is expected, discharge through the dam may be varied, within the limits of the project design, to optimize sulfur removal from the system.

Toxicity studies with hydrogen sulfide have shown that static bioassay studies are not satisfactory. We recommend the use of a continuous-flow system (L. L. Smith, Jr., pers. comm., also suggested this system) with a hydrogen sulfide generator supplying the gas. Stabilization of pH and provision for varying dissolved oxygen concentration would be useful additions to such a system. We selected an anaerobic system because we felt that this best represented field situations. Colby and Smith (1969) present a very workable continuous-flow system, however a vacuum system for removing oxygen, rather than a nitrogen stripping column, would be preferred.

A recommended field study would be the vertical distribution of Chaoborus larvae in two systems, one with and one without hydrogen sulfide in the hypolimnion.

SUMMARY

1. The sources and annual cycle of sulfur were studied in the Lake Shelbyville Basin, Illinois, from 6 April 1972 through 28 March 1973.

2. Sources of sulfur in the basin included rainfall (3.34×10^4 kg $year^{-1}$), surface runoff (2.05×10^7 kg $year^{-1}$), and pollution (4.56×10^6 kg $year^{-1}$). Decomposition of inundated organic matter and solution of inorganic sulfur compounds from the inundated basin were considered one-time sources (first year of impoundment). Exchange between groundwater and the lake was considered to be in long-term equilibrium.

3. Regression analysis of the relationship between sulfate loading and discharge for both stations I and X, figures 2 and 3, respectively, provided an accurate means of calculating sulfate concentration from discharge measurements.

4. During the study year, 2.51×10^7 kg of sulfur entered Lake Shelbyville. Of this, 2.01×10^7 kg (80.1%) flowed out through the dam, 3.35×10^6 kg (13.3%) remained in the lake due to a net gain in water storage, and 1.65×10^6 kg (6.6%) was either assimilated by the biota or, in part, lost to the groundwater.

5. Reduction of sulfate and the precipitation of ferrous sulfide lowered the sulfate concentration near the mud-water interface during summer stagnation.

6. During summer, 1971, sulfate-reducing bacteria produced substantial quantities of hydrogen sulfide in the lake. Purple sulfur bacteria formed a bacterial plate at the bottom of the oxygenated zone and oxidized this sulfide. During summer, 1972, sulfate-reducing bacteria were present in much-reduced numbers, perhaps due to the lack of sufficient organic matter. Green sulfur bacteria were present and oxidized sulfides in equilibrium with the reduction process. Free hydrogen sulfide was not detectable.

7. Static bioassay studies of Chaoborus larvae yielded a minimum effect level for sulfide of 0.64 mg liter^{-1} (as S) for a 4-hour test.

8. Avoidance experiments with Chaoborus larvae and pupae indicated that pupae respond more rapidly to sulfide than do larvae. Relativly brief exposure to a low (0.8 mg liter^{-1} as S) sulfide concentration was often sufficient to kill or immobilize (thus preventing escape and ultimately resulting in death) a substantial percentage of the test organisms.

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